



A revised taxonomy and estimate of species diversity for western North American *Lampetra*

K. J. Carim · D. C. Larson · J. M. Helstab ·
M. K. Young · M. F. Docker

Received: 1 April 2022 / Accepted: 2 February 2023 / Published online: 14 February 2023

This is a U.S. Government work and not under copyright protection in the US; foreign copyright protection may apply 2023

Abstract Lampreys are an ancient lineage of jawless fish for which the relationships among some lineages are uncertain. We addressed these information gaps for *Lampetra* species with a focus on western North America. Phylogenetic analysis using all publicly available sequences of two mitochondrial genes—*cyt b* and *COI*—supported designation of western North American

Lampetra as a distinct genus from *Lampetra* in Europe, Asia, and eastern North America. Species delimitation analysis of *cyt b* sequences identified seven species, only three of which are currently recognized. *Lampetra ayresii* and *L. richardsoni*, regarded as separate under the “paired species concept,” were not genetically distinct; we recommend synonymizing *L. richardsoni* with *L. ayresii* because the latter has precedence, yet continuing to recognize each ecotype using its current common name. Other than the widespread *L. ayresii* clade, all other species exhibited limited ranges often restricted to one or two locations. Within headwaters of the Willamette River, *L. ayresii* demonstrated strong genetic structure and lack of gene flow among subbasins, indicating that the subbasin scale may represent an appropriate management unit. However, the appropriate management scale in other areas may differ based on evolutionary histories and gene flow. Subsequent work is needed across western North America to better delineate the ranges of the aforementioned species and to evaluate whether other unrecognized taxa in this lineage may be present.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10641-023-01397-y>.

K. J. Carim (✉)
Aldo Leopold Wilderness Research Institute, U.S. Forest Service, Rocky Mountain Research Station, Missoula, MT, USA
e-mail: kellie.carim@usda.gov

K. J. Carim · M. K. Young
U.S. Forest Service, Rocky Mountain Research Station, National Genomics Center for Wildlife and Fish Conservation, Missoula, MT, USA
e-mail: michael.k.young@usda.gov

D. C. Larson · J. M. Helstab
U.S. Forest Service, Middle Fork Ranger District, Westfir, OR, USA
e-mail: douglas.larson@usda.gov

J. M. Helstab
e-mail: joseph.helstab@usda.gov

M. F. Docker
Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
e-mail: margaret.docker@umanitoba.ca

Keywords Willamette River · Phylogenetics · Species delimitation · Conservation · Lamprey · Petromyzontidae

Introduction

Lampreys are jawless aquatic vertebrates in the order Petromyzontiformes. They are globally distributed,

with two families, Geotriidae and Mordaciidae, in the Southern Hemisphere, and one family, Petromyzontidae, in the Northern Hemisphere (Potter et al. 2015). There is consensus on the placement of taxa within these families (Gill et al. 2003; Renaud 2011), but their systematics at lower levels of classification, such as the relationships among genera and assignment of species within them, are contentious and fluid (Potter et al. 2015; Docker and Hume 2019). This is in part because lampreys lack many of the morphological characteristics used in taxonomic studies of bony fishes, and those few putatively diagnostic characters are often only expressed in the adult life stage (Renaud 2011). At an even finer scale, recent molecular analyses cast doubt on the accuracy of separating some lampreys as distinct species. A recurring pattern among lamprey taxa is the presence of species complexes whose members are often described as paired or stem-satellite species (Docker 2009). The members of a complex are often morphologically indistinguishable during their extended larval stages (Zanandrea 1959; Potter 1980) but develop striking anatomical and behavioral differences as juveniles and adults. Each complex consists of a migratory (anadromous or potamodromous) species with a parasitic, predatory, or necrophagous phase prior to sexual maturity and one or more closely related species that presumably evolved from the former, which as adults either remain in freshwater, do not feed, or both (Docker 2009; Docker and Potter 2019). Although the present taxonomy of lampreys reflects the belief that phenotypic diversity in life history expression is grounds for species designation (Bailey 1980; Potter 1980), this interpretation has not been universally accepted among lamprey researchers (McPhail and Lindsey 1970; Espanhol et al. 2007; Kucheryavyy et al. 2016a, 2016b) and is rarely supported by genetic information. For example, Docker et al. (2012) found that in one species pair—the parasitic silver lamprey *Ichthyomyzon unicuspis* and non-parasitic northern brook lamprey *I. fossor*—putative specimens of both species collected from the same rivers could not be differentiated using either mitochondrial (mt) DNA sequences or microsatellite loci (see also April et al. 2011). Similarly, many of the species within the genus *Entosphenus* lack diagnostic differences in their mtDNA sequences despite different migratory or feeding life histories (Lorion et al. 2000; Lang et al. 2009; Taylor et al. 2012).

There is also evidence that species designations based on phenotype overlook cryptic taxa. For example, Yamazaki et al. (2006) analyzed mtDNA sequences and allozymes of specimens of *Lethenteron* from across eastern Asia. Although the authors argued that Siberian brook lamprey *Lethenteron kessleri* should be regarded as a synonym for Far Eastern brook lamprey *Lethenteron reissneri*, they also observed two groups presumed to be *Lethenteron reissneri* that were highly genetically divergent; one group appeared to represent a new species within this genus (*Lethenteron* sp. N), and the other group likely constituted a new species unaffiliated with *Lethenteron* (albeit labeled *Lethenteron* sp. S as a placeholder). Likewise, Boguski et al. (2012) found that morphologically similar but geographically disparate populations of *Lampetra* sp. were genetically divergent and hypothesized that some may represent undescribed species.

Many of the aforementioned issues are evident among species of *Lampetra*. The type species for this genus—European river lamprey *Lampetra fluviatilis*—and several congeners in Europe are distantly related to those in North America (Lang et al. 2009; Boguski et al. 2012), so much so that the lone member from eastern North America has been regarded by some as belonging to a different genus (*Okkelbergia*; Hubbs and Creaser 1922). Similarly, phylogenetic analyses suggest that *Lampetra* of western North America may also constitute a separate genus (Docker et al. 1999; Blank et al. 2008; Lang et al. 2009). In western North America, the current taxonomy recognizes four species: (1) western river lamprey *Lampetra ayresii*, an anadromous, parasitic species with a disjunct distribution restricted to large rivers and their estuaries from Alaska to California; (2) western brook lamprey *Lampetra richardsoni*, a non-parasitic freshwater species (but see Beamish 1987), considered a species pair with *Lampetra ayresii* and sharing a similar but more continuous range; (3) Kern brook lamprey *Lampetra hubbsi*, originally considered to belong to *Entosphenus*, with the same life history as *L. richardsoni* but found only in the San Joaquin River basin in central California (Moyle 2002; Potter et al. 2015); and (4) Pacific brook lamprey *Lampetra pacifica*, also maturing as a non-parasitic species in freshwater that was originally thought to be widely distributed in Oregon and California (Vladykov 1973), then synonymized with *L. richardsoni* (Robins

et al. 1991), and most recently taxonomically resurrected as a distinct species and thought to inhabit an undefined portion of the Columbia River basin (Reid et al. 2011) that apparently does not include Washington (Wydoski and Whitney 2003).

Several approaches could reduce the uncertainty about the taxonomic status of western North American *Lampetra* within Petromyzontidae. Although phylogenetic analyses have included thorough treatments of particular genera or regions (Yamazaki et al. 2006; April et al. 2011; Boguski et al. 2012; Li 2014; White 2014) and broad overviews of all genera (Lang et al. 2009), no analysis has taken advantage of all publicly available sequences for any gene region to place *Lampetra* of western North America in their evolutionary context relative to other lampreys. Moreover, formal molecular species delimitation has not been undertaken for any genus of lamprey. Finally, understanding to what degree geography dictates population structure within species is important for guiding management actions.

Clarifying these issues for *Lampetra* in western North America is more than a taxonomic issue. In 2004, the US Fish and Wildlife Service denied a petition for listing *Lampetra ayresii* and *Lampetra richardsoni* (as well as Pacific lamprey *Entosphenus tridentatus*) in the western continental USA under the Endangered Species Act (US Fish and Wildlife Service 2004). In their decision, the agency concluded that there was no evidence for a widespread decline in either *Lampetra* species but regarded each as having a broad distribution from Alaska to California. If these taxa constitute a species complex, however, it may be worthwhile to evaluate the conservation status of all of its members. To help fill this information gap, we developed a global phylogeny of all members of Petromyzontidae to assess the phylogenetic position of the western North American *Lampetra* (Objective 1). We used all available sequences of two mitochondrial gene regions in public sequence repositories (that met minimum length and quality thresholds), as well as sequences of new specimens from much of the Willamette River basin in Oregon. Because sequences for one of these gene regions were taxonomically comprehensive, we performed molecular species delimitation among samples of western North American *Lampetra* using a variety of

approaches to generate hypotheses about the number, identity, and distribution of species (Objective 2). We refined our estimates of the distribution of some of these taxa by using an even larger dataset and performing molecular specimen assignment. Finally, we assessed haplotype diversity and evaluated fine-scale genetic structure of *Lampetra* among adjacent subbasins of the Willamette River basin to understand population boundaries and identify units for management and conservation (Objective 3).

Methods

Study area

Data collection for this study took place across six, 8-digit hydrologic units (subbasins averaging ~1125 km²; US Geological Survey 2004) located in the Willamette River basin, Oregon, USA: the Upper Willamette, North Santiam, South Santiam, McKenzie, Middle Fork Willamette, and Coast Fork Willamette River subbasins (Fig. 1). Thirteen major hydroelectric dams in this basin create barriers to upstream fish passage, preventing migratory species from reaching hundreds of kilometers of spawning and rearing habitat in the upper subbasins. Consequently, migratory fish species, including *Entosphenus tridentatus*, were largely extirpated from their historical ranges in these subbasins. Information on species identity and distributions of *Lampetra* throughout the Willamette River basin is limited. Fish collections dating back to 1951 suggest the presence of *Lampetra pacifica* or *Lampetra richardsoni* throughout the Willamette River, including our study area (see below; Table S1; Fishnet2 Portal, www.fishnet2.org, accessed on July 23, 2022 and September 23, 2022). Reid et al. (2011) used paired morphological and genetic analysis to confirm the presence of *Lampetra richardsoni* in the Tualatin subbasin and *Lampetra pacifica* in the Clackamas subbasin (both tributaries to the lower main stem Willamette River). No other studies have attempted to identify species of *Lampetra* or their distribution and population boundaries in the Willamette River basin.

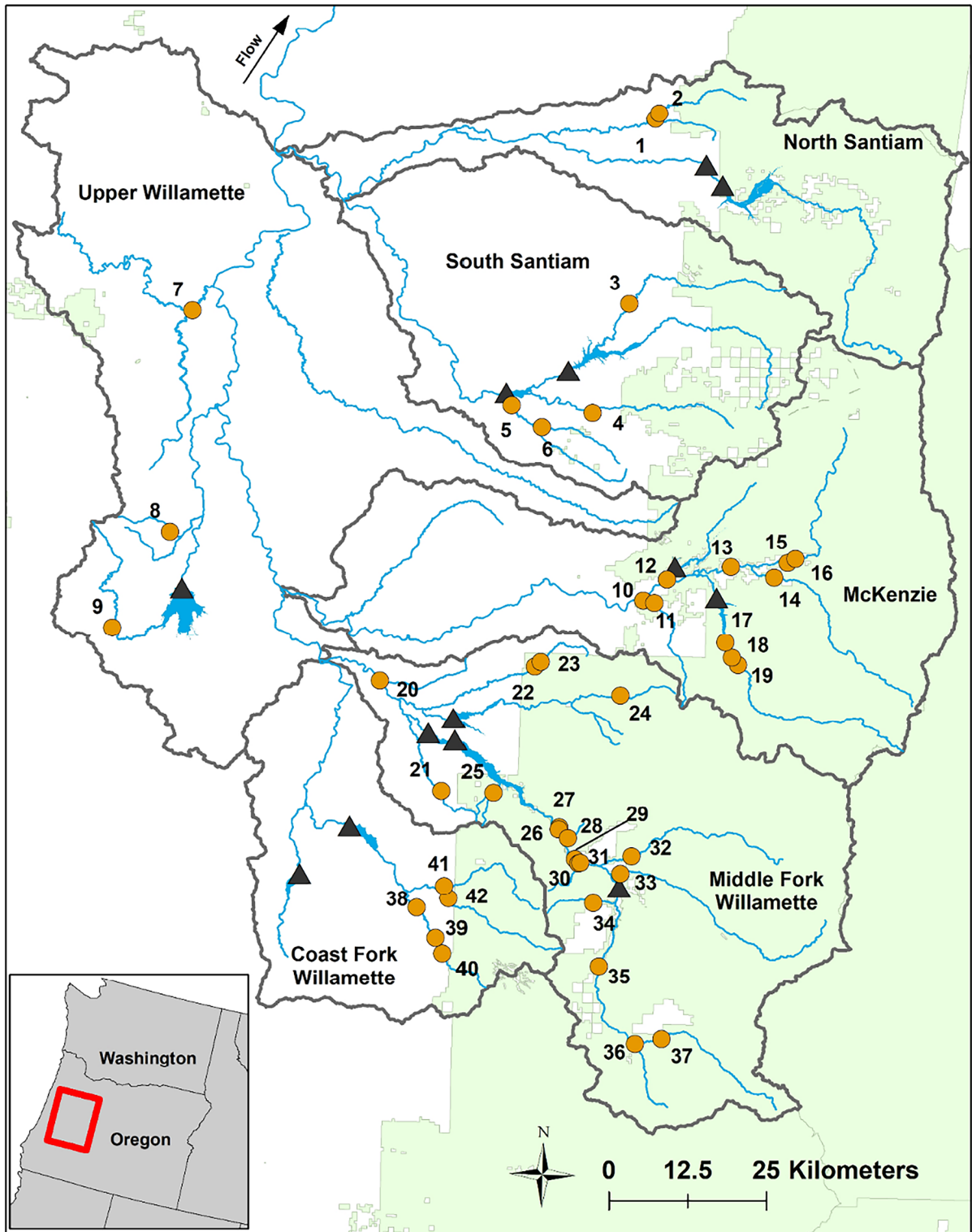


Fig. 1 Locations of lamprey samples collected in this study. Labels denote the subbasin (4th-level hydrologic unit). Numbers correspond to Map ID in Table S1. Black triangles rep-

resent locations of major hydroelectric dams; green shading represents the US National Forest boundaries

New sample collection

New collections were obtained as part of a larger study to determine the occupancy of lampreys throughout subbasins of the Willamette River basin on the Willamette National Forest. Survey locations were selected using a general random tessellation stratified (GRTS) sampling regime structured at the level of 12-digit hydrologic units (sub-watersheds). Surveys targeted three 100-m segments of floodplain channels as well as perennial streams with an average slope less than 2.5%. Only surveys where lampreys were observed were included in this study. To increase the sample size and geographic scope of this study, additional surveys were conducted outside of the Willamette National Forest. These additional sites were selected based on accessibility (e.g., road crossings) and targeted shallow, low-gradient reaches (< 2.5% stream slope) considered suitable for lamprey.

Between April 2015 and October 2017, larval (and occasionally adult) lampreys were captured at 42 locations in the Willamette River basin using a Smith-Root LR-24 backpack electrofisher (Table S1, Fig. 1). Specimens were sedated using tricaine methanesulfonate diluted in stream water, and tissue samples were collected from each individual. Tissues were preserved individually in vials filled with 95% ethanol and stored at room temperature. Collections were made following the Guidelines for the Use of Fishes in Research, Oregon Department of Fish and Wildlife Scientific Taking Permit 4(d) Permit # 22632 following methods outlined in Jenkins et al. (2014).

During field surveys, *Lampetra* were distinguished from *E. tridentatus* based on pigmentation of the caudal ridge and caudal fin (Goodman et al. 2009). Although we did not attempt to identify *Lampetra* to species in the field, we expected that most specimens would be *Lampetra richardsoni* or *Lampetra pacifica* (Reid et al. 2011; Boguski et al. 2012; Potter et al. 2015). We did not expect to find putative *Lampetra ayresii* because this form is migratory, typically found in coastal systems, and has never been documented in our study area.

DNA extraction and sequencing

We used the Qiagen DNeasy® Blood and Tissue Kit to extract DNA from up to eight individuals from a given sampling site (Table S1) and stored the DNA

at −20 °C until sequenced. A 988-bp segment of the cytochrome *b* (*cyt b*) gene and a 1337-bp segment of the cytochrome c oxidase subunit I (*COI*) gene was amplified from each sample using existing or newly developed primers (Table S2; Boguski et al. 2012). The 40-μl amplification reactions contained 4 μl (~4–20 ng) DNA, 4 μl of 10× PCR buffer, 4 μl MgCl₂ (2.5 mM), 1 μM of each primer, 200 μM each dNTP, 25 μg BSA, and 1 Unit Titanium® Taq DNA Polymerase (Takara Bio USA, Inc.), with the remainder consisting of PCR-grade distilled water. Segments of the *cyt b* gene were amplified under the following conditions: 2 min at 94 °C, 10 × (2 min at 94 °C, 60 s at 60 °C, 2 min at 72 °C), 10 × (2 min at 94 °C, 60 s at 58 °C, 2 min at 72 °C), 10 × (2 min at 94 °C, 60 s at 55 °C, 2 min at 72 °C), 5 min at 72 °C, then held at 12 °C. Segments of the *COI* gene were amplified under the following conditions: 12 min at 95 °C, 35 × (60 s at 95 °C, 60 s at 55 °C, 90 s at 72 °C), 5 min at 72 °C, then held at 12 °C. The PCR products were cleaned with Exo-SAP-IT (Affymetrix), and DNA sequence data were obtained using the Big Dye kit and the 3700 DNA Analyzer (ABI; Eurofins Genomics, Louisville, KY). Sequences were viewed and aligned with Sequencher (Gene Codes Corp. MI). We used MEGA version 7 (Kumar et al. 2016) to translate all sequences into amino acids to verify there were no stop codons or nonsense bases which would indicate a sequencing error or the presence of nuclear copies of mitochondrial DNA.

Sequence datasets

To understand the phylogenetic position of western North American *Lampetra* within the Petromyzontidae, we created two sequence datasets. The “*cyt b* dataset” comprised the 988-base *cyt b* sequences obtained in this study ($n = 134$) and all publicly available sequences (in GenBank; $n = 388$) representing species in Petromyzontidae (Table S3; Table S4). We excluded public sequences that did not completely overlap our sequences or that contained ambiguous or missing bases; sequences longer than 988 bp were trimmed to ensure consistent coverage across all sequences in the dataset. Similarly, the “*COI* dataset” comprised 585-base *COI* sequences from our specimens and an additional 393 publicly available sequences, screened for length and quality (Table S3; Table S4). For both datasets, we used

all publicly available sequences (*cyt b*, $n = 6$; *COI*, $n = 48$) of species of *Geotria* and *Mordacia*, representing the remaining two families in Petromyzontiformes, as outgroups. All public sequence labels were updated to reflect the presently accepted taxonomy following Fricke et al. (2022; e.g., sequences labeled as *Lampetra tridentata* were updated to *Entosphenus tridentatus*). Apparent misidentified specimens were ignored, except one *COI* sequence identified as *Lampetra ayresii* (JQ354155), but which was identical to sequences of *Entosphenus tridentatus* and thus relabeled.

We created two datasets to address the objective related to species delimitation and geographic distribution. For species delimitation within the western North American *Lampetra*, we created the “species delimitation dataset.” This dataset used the *cyt b* dataset restricted to representatives of *Lampetra* from western North America ($n = 294$) and a single sequence of *Entosphenus tridentatus* as an outgroup. Finally, to more precisely describe the geographic distribution of delimited species, we created a “specimen assignment” dataset that also included the shorter sequences (295–438 bases; $n = 205$) excluded from the species delimitation dataset (Table S3).

Finally, to understand genetic diversity and structure of *Lampetra* among subbasins in our study area, we created a “concatenated” dataset representing only new samples collected in this study by concatenating a 1337-base segment of the *COI* gene and a 988-base segment of the *cyt b* gene to create a single 2325-base sequence for each individual. Sequences were concatenated using Geneious 10.0.6 (<http://www.geneious.com>, Kearse et al. 2012). Prior to analysis, we reduced sequences in the species delimitation and concatenated datasets to representative haplotypes.

Objective 1: phylogenetic position of western North American *Lampetra*

To better understand the systematics of *Lampetra* from western North America within Petromyzontidae, we used the *cyt b* and *COI* datasets to estimate maximum likelihood phylogenetic trees in IQ-TREE 2.1.1 (Minh et al. 2020) and implemented via the CIPRES gateway (<https://www.phylo.org/>). Initially, we removed the outgroups from each dataset, assigned three preliminary partitions based on codon position (see Bofkin and Goldman 2007), then selected

edge-linked partitions and the TESTMERGE setting to determine the best-fitting evolutionary model (as measured by BIC) for each partition (Table S5). We then added the outgroup sequences and constructed a consensus maximum-likelihood tree for each dataset, assigning support values based on 1000 ultrafast bootstrap replicates. The final trees were visualized and edited using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree>) and Inkscape v1.0.1 (<http://inkscape.org>).

Objective 2: species delimitation and specimen assignment in western North American *Lampetra*

We used five methods of single-locus species delimitation to generate species hypotheses within *Lampetra* from western North America using the species delimitation dataset. We recognized species under the phylogenetic species concept, which relies on reciprocal monophyly among lineages (Nixon and Wheeler 1990), further modified by a distance threshold often typical of interspecific differences among fishes (Ward 2009). We began by constructing a maximum likelihood tree for this dataset following the approach described for Objective 1. In the first analysis, we input this tree into the online version of maximum-likelihood, multi-rate Poisson tree processes (mPTP; <https://mptp.h-its.org/#/tree>; Kapli et al. 2017), which examines branch lengths to estimate the shift from intraspecific to interspecific divergence and can provide a liberal estimate of species diversity relative to other methods (Zhang et al. 2013). Second, we constructed statistical parsimony networks (SPN) with a 95% parsimony limit in TCS v1.21 (Clement et al. 2000) and visualized these using tcsBU (<https://cibio.up.pt/software/tcsBU>; Santos et al. 2015). When applied to datasets with multiple taxa, the number of unconnected networks constitutes a conservative estimate of species diversity (Hart and Sunday 2007). Third, we used the online version (<https://bioinfo.mnhn.fr/abi/public/asap/#>) of ASAP (Assemble Species by Automatic Partitioning; Puillandre et al. 2021), for which pairwise genetic distances among haplotypes are used to distinguish between intraspecific variation and interspecific divergence and a scoring system to identify the best-fitting set of partitions (i.e., candidate species) based on both the probability of panmixia in a given partition and genetic distances within and among partitions. The set of partitions

with the lowest score is deemed the most likely number of species. We adopted the default values and distances based on the K80 substitution model because of its similarity to the traditional distance metric used in sequence-based analyses (Ratnasingham and Hebert 2013). The analysis was run 10 times with different initial seeds, for which the highest-scoring set of partitions only changed once. Because the approach is similar to ABGD (Puillandre et al. 2012), we assumed that species counts would be conservative relative to other methods (Puillandre et al. 2021). Fourth, we reexamined the maximum-likelihood tree from the first step and interpreted bootstrap values > 85 as strong support (Minh et al. 2013) for a candidate species identified by one of the preceding methods. Finally, we used MEGA 7 (Kumar et al. 2016) to examine pairwise genetic distances, expressed as p-distance, among these candidate species. We contrasted the maximum intraspecific distances with the minimum interspecific distances. If the latter exceeded ~2% and was larger than the former (i.e., a barcode gap was present; Ratnasingham and Hebert 2013), we interpreted this as supporting the designation of a candidate species.

Due to the limited representation of the *COI* gene in public databases, we did not perform a species delimitation analysis using this gene. However, we did compare placement of *Lampetra* sp. from western North America in the *COI* maximum likelihood tree (above) to the *cyt b* tree to broadly look for any apparent differences in species placement.

Finally, to assign specimens to putative species, we built a *cyt b* neighbor-joining tree (in MEGA 7 using the number of base pair of differences) using the specimen assignment dataset.

Objective 3: haplotype diversity and genetic structure among lampreys in the Willamette River basin

To assess haplotype diversity among lamprey in the Willamette River basin (Objective 3), we used the concatenated dataset to create statistical parsimony networks following the method described in Objective 2 above. Unlike phylogenetic trees, statistical parsimony networks are unrooted and do not assume bifurcating divergence (Clement et al. 2000). As a result, they are useful for exploring haplotype relatedness at the population level, particularly when the number of variable sites may be low and parsimony

methods will be unable to resolve branching patterns with high levels of support (Clement et al. 2000).

To look for genetic substructure in *Lampetra* spp. in the Willamette River basin, we grouped individual sequences from the concatenated dataset based on subbasin of capture. We then used MEGA 7 to calculate average p-distance within and among individuals grouped by subbasin. We performed an analysis of molecular variance (AMOVA) in R (R Core Team 2020) using the `poppr.amova()` function in the package “poppr” to assess population differentiation at the subbasin level (Kamvar et al. 2014). Significance for the AMOVA was assessed at $\alpha \leq 0.05$.

Results

Objective 1: phylogenetic position of western North American *Lampetra*

The *cyt b* maximum likelihood tree (Fig. 2; Fig. S1) robustly supported (100% bootstrap support) two clades representing the Petromyzontinae subfamily (*Ichthyomyzon*, *Petromyzon*, and *Caspiomyzon*) and the Lampetrinae subfamily (*Entosphenus*, *Tetrapleurodon*, *Lethenteron*, *Lampetra*, and *Eudontomyzon*). Within Lampetrinae, five clades also had bootstrap support of 100: (1) three specimens identified as Arctic lamprey *Lethenteron camtschaticum* or *L. reissneri* (equivalent to the aforementioned *Lethenteron* sp. S; Yamazaki et al. 2006; Lang et al. 2009; Li 2014); (2) *Entosphenus* plus *Tetrapleurodon*; (3) all remaining *Lethenteron* (save one; see below) plus Korean lamprey *Eudontomyzon morii*; (4) western North American *Lampetra*; and (5) all remaining *Eudontomyzon* and *Lampetra* and Western Transcaucasian brook lamprey *Lethenteron ninae*. This last clade was further subdivided into three additional highly supported lineages: (1) *Eudontomyzon*; (2) least brook lamprey *Lampetra aepyptera* from eastern North America; and (3) all remaining European and Asian *Lampetra* plus *Lethenteron ninae*.

The topology of the *COI* maximum likelihood tree (Fig. 2; Fig. S2) was remarkably similar to that of the *cyt b* tree but showed even greater support at intermediate branches. Bootstrap support for subfamilies Petromyzontinae and Lampetrinae was 99 and 100%, respectively, and relationships within Petromyzontinae (*Petromyzon* + *Ichthyomyzon* sister

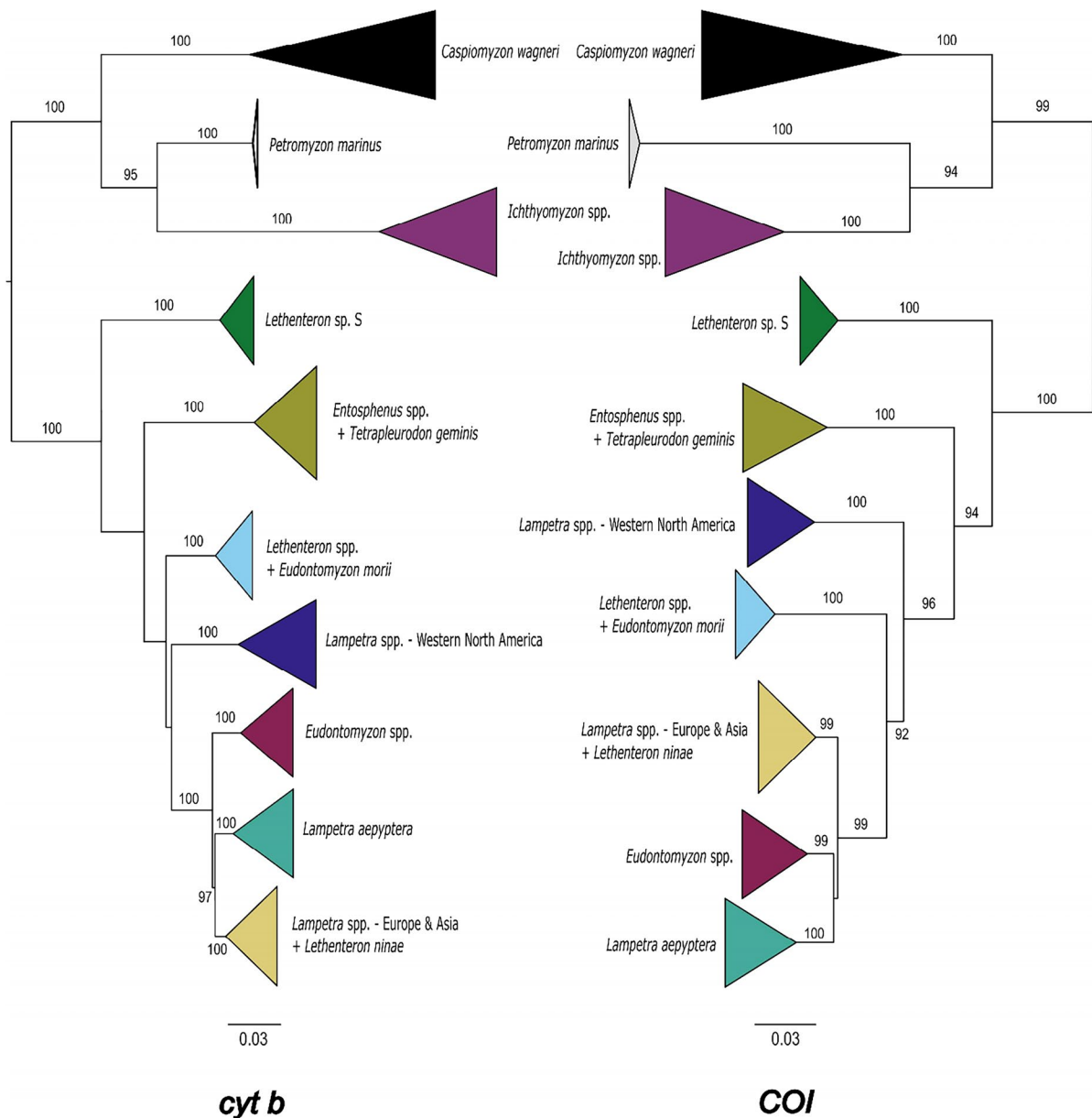


Fig. 2 Maximum likelihood trees showing the phylogenetic relationship of Northern Hemisphere lampreys at the *cyt b* gene and *COI* genes. Bootstrap values are shown for branches

with $\geq 85\%$ support. See Figures S1 and S2 for an expanded version of each tree displaying all sequences in each dataset

to *Caspiomyzon*) were identical to those observed in *cyt b*. Within Lampetrinae, membership within the same five clades was identical to that in the *cyt b* phylogeny and well supported. Intermediate branches relating these clades differed slightly from the *cyt b* tree but also had strong ($> 90\%$) support. Clade membership, in descending order of divergence, was

(1) “*Lethenteron* sp. S”; (2) *Entosphenus* + *Tetrapleurodon*; (3) western North American *Lampetra*; (4) *Lethenteron* (save one) and *Eudontomyzon morii*; and (5) remaining members of *Lampetra*, *Eudontomyzon*, and *Lethenteron ninae*. Division of the last clade into the same three additional clades seen with *cyt b* was also strongly supported (bootstrap support

≥ 99%): (1) *Lampetra* from Europe and Asia, along with *Lethenteron ninae*; (2) all remaining *Eudontomyzon*; and (3) *Lampetra aepyptera* in eastern North America.

Objective 2: species delimitation and specimen assignment in western North American *Lampetra*

In the 988-bp species delimitation dataset, we observed 160 variable nucleotides sites resulting in 67 haplotypes (Table S3). The mPTP and statistical

parsimony networks delimited seven candidate species (Figs. 3 and 4), the first six of which had limited distributions: (1) *Lampetra pacifica* from the Willamette River basin below Willamette Falls (*cyt b* H63 and H64); (2) *Lampetra hubbsi* from the San Joaquin River basin (*cyt b* H54) and one individual from Paynes Creek in the Sacramento River basin, California (*cyt b* H55); (3) specimens from the Siuslaw River, Oregon (*cyt b* haplotypes H56, H57, and H58); (4) specimens from Fourmile Creek in the Klamath River basin, Oregon (*cyt b* haplotypes H59,

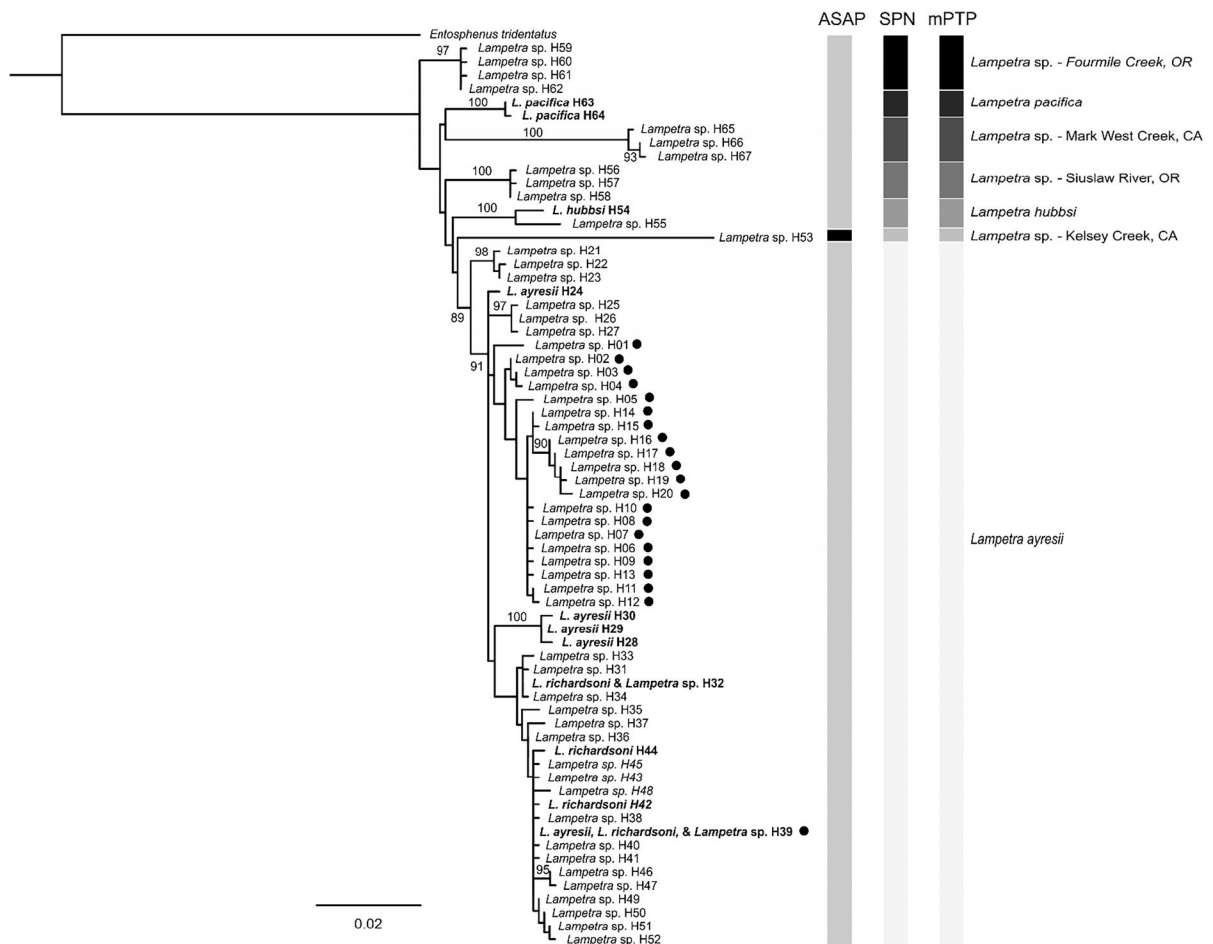


Fig. 3 Maximum likelihood tree displaying relationships among *Lampetra* spp. of western North America at the *cyt b* gene. Tip labels correspond to *cyt b* haplotypes in Table S4. Haplotypes in bold include at least one sequence identified to the species level. Bootstrap values are shown for branches with ≥ 85% support. Black dots denote haplotypes observed in the headwater subbasins of the Willamette River (this project's study area). Bars to the right of the phylogenetic trees denote

species groups identified by ASAP (Assemble Species by Automatic Partitioning; $n = 2$ species), SPN (statistical parsimony networks; $n = 7$ species), and mPTP (multi-rate Poisson tree processes; $n = 7$ species). Labels to the right correspond to the seven species groups identified through species delimitation analysis. All haplotypes identified as *L. richardsoni* are included in the *L. ayresii* species group

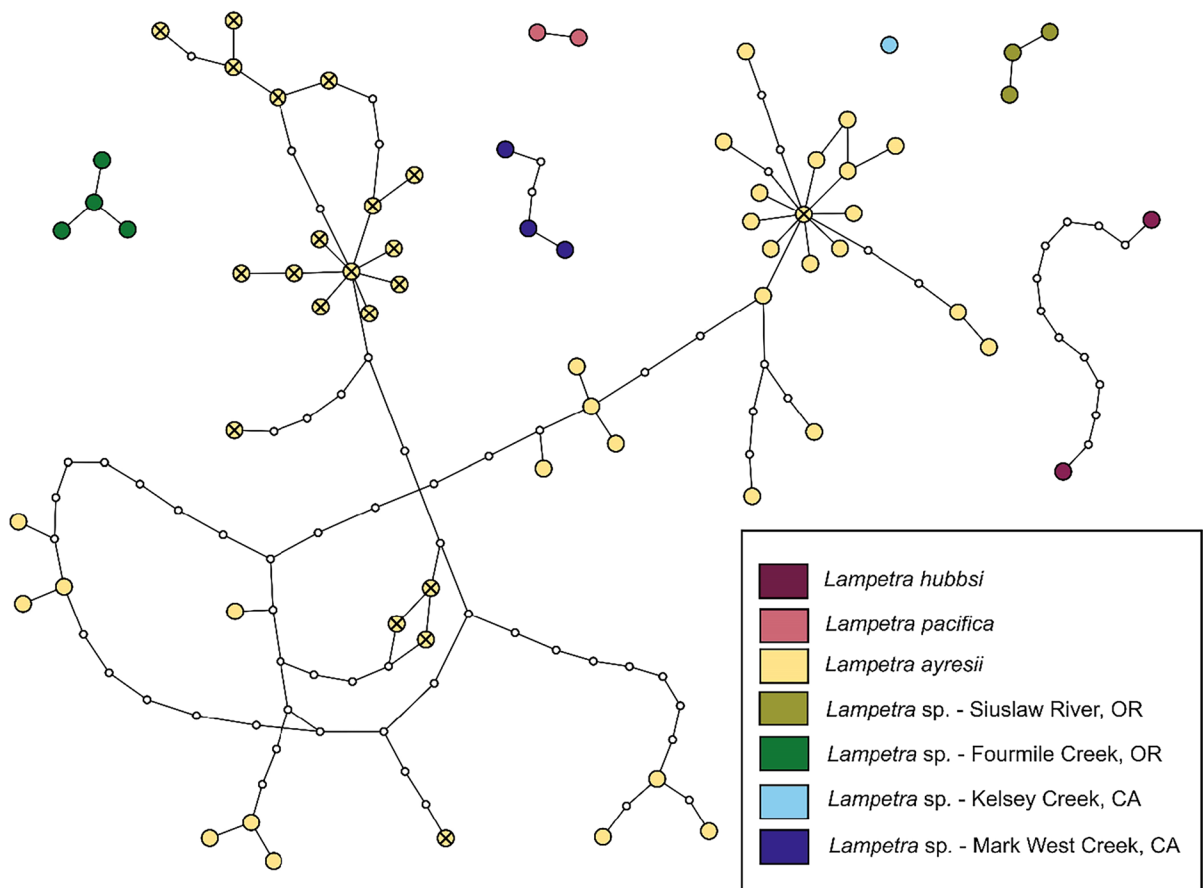


Fig. 4 Statistical parsimony network (SPN) showing relatedness among *cyt b* haplotypes of *Lampetra* sp. in western North America. Nodes represent unique haplotypes and are color-coded based on putative species groups. Nodes marked with a cross denote haplotypes observed in headwater subbasins of the Willamette River. Small white nodes show transition-

ary haplotypes that were not observed in the current dataset. Segments between nodes represent a single base pair mismatch from each connected node. All haplotypes representing specimens labeled as *L. richardsoni* are included in the *L. ayresii* species group

H60, H61, and H62); (5) specimens from Mark West Creek in the Russian River basin, California (*cyt b* haplotypes H65, H66, and H67); and (6) specimens from Kelsey Creek, a tributary to Clear Lake, California (*cyt b* H53). These six candidate species were supported in the maximum likelihood tree with 97–100% bootstrap support, except for those from Kelsey Creek, which did not receive an estimate because it was represented by only one haplotype. The seventh candidate species included 52 haplotypes (*cyt b* H01–H52) variously identified as *Lampetra ayresii*, *Lampetra richardsoni*, and *Lampetra* sp. and was supported with 89% bootstrap support in the maximum likelihood tree. Within this lineage, haplotypes identified as *Lampetra richardsoni* and *Lampetra*

ayresii were polyphyletic (Fig. 3). This candidate species contained all 21 *Lampetra* haplotypes from our study area in the Willamette River basin, 20 of which grouped into a single subtree. The 21st haplotype (*cyt b* H39) represented three individuals from Muddy Creek in the Upper Willamette River basin, along with an additional 36 individuals from 20 waterbodies across Alaska, British Columbia, Washington, and Oregon.

The ASAP analysis was more conservative than the others, with support for two candidate species (Fig. 3). Here, only *Lampetra* from Kelsey Creek, California, was delimited as a single candidate species; all others were pooled into a single candidate taxon. The next best fitting model in 9 of 10 analyses

also delimited *Lampetra* in Mark West Creek, California, as a candidate species.

Divergence among all *Lampetra* haplotypes in the species delimitation dataset ranged from 0.1 to 6.4% (Table 1). When grouped into the consensus seven candidate species, six of these exhibited intraspecific divergence $\leq 1.21\%$ and a barcode gap (sensu Ratnasingham and Hebert 2013) exceeding 1.70% (minimum interspecific distance observed was 1.72% between *Lampetra ayresii* and the Siuslaw River lineage). The maximum intraspecific divergence (2.33%) within the highly diverse and widespread seventh candidate species (which included specimens identified as *Lampetra ayresii* and *Lampetra richardsoni*) exceeded its minimum interspecific divergence for all but two of the candidate species.

The *COI* maximum likelihood tree (Fig. S2) had limited coverage of *Lampetra* from western North America. However, it also resolved *Lampetra hubbsi* and *Lampetra pacifica* as highly supported (bootstrap support $\geq 99\%$) clades. These had an uncertain relation to other specimens of *Lampetra* from Oregon and Washington that were likely to represent the widespread, diverse *L. ayresii* complex from the species delimitation analysis.

The sequences included for specimen assignment ($n = 205$) represented 33 river drainages ranging from British Columbia to California, with most in Washington (Table S3). All specimens unambiguously assigned to one of the delimited taxa. Specifically, one sequence (AF177958) represented specimens collected from the Merced and Kings Rivers in California and assigned to *Lampetra hubbsi*; all other sequences assigned to *Lampetra ayresii* (Fig. S3; Table S3).

Table 1 Genetic differences (measured as p-distance and expressed as a percentage) within and among candidate species of western North American *Lampetra*. Values on the diagonal (in bold) are maximum intraspecific differences; values below the diagonal are minimum interspecific differences. Specimens

	<i>L. pacifica</i>	<i>L. hubbsi</i>	Siuslaw River, OR	Fourmile Creek, OR	Mark West Creek, CA	Kelsey Creek, CA	<i>L. ayresii</i>
<i>L. pacifica</i>	0.10						
<i>L. hubbsi</i>	2.43	1.21					
Siuslaw River, OR	2.13	2.63	0.20				
Fourmile Creek, OR	2.23	2.43	2.02	0.20			
Mark West Creek, CA	3.85	4.66	3.74	3.85	0.40		
Kelsey Creek, CA	4.96	4.76	4.96	5.16	6.38	—	
<i>L. ayresii</i>	1.82	2.13	1.72	1.82	3.74	4.45	2.33

from Kelsey Creek were represented by one haplotype, so the intraspecific difference was not estimated. The *L. ayresii* species group includes both anadromous/parasitic and resident/non-parasitic ecotypes, commonly known as western river lamprey and western brook lamprey, respectively

Table 2 Average pairwise genetic distance (p-distance, expressed as a percentage) of *Lampetra* within and among subbasins in the Willamette River basin. Average within-group genetic distance is listed on the diagonal in bold

	Coast Fork Willamette	McKenzie	Middle Fork Willamette	North Santiam	South Santiam	Upper Willamette
Coast Fork Willamette	0.09					
McKenzie	0.45	0.14				
Middle Fork Willamette	0.38	0.17	0.06			
North Santiam	0.53	0.55	0.48	0.01		
South Santiam	0.39	0.16	0.13	0.49	0.06	
Upper Willamette	0.47	0.77	0.69	0.79	0.70	0.66

Objective 3: haplotype diversity and genetic structure among lampreys in the Willamette River basin

In the concatenated dataset, divergence among *Lampetra* captured in headwater subbasins of the Willamette River basin ranged from 0.1 to 1.6% across

71 variable nucleotide sites resulting in 34 haplotypes (Table S3, Table S6). The statistical parsimony network estimated from the concatenated dataset revealed substantial geographic structure (Fig. 5). Only one haplotype (W) was observed in more than one subbasin. Three haplotypes in the Upper

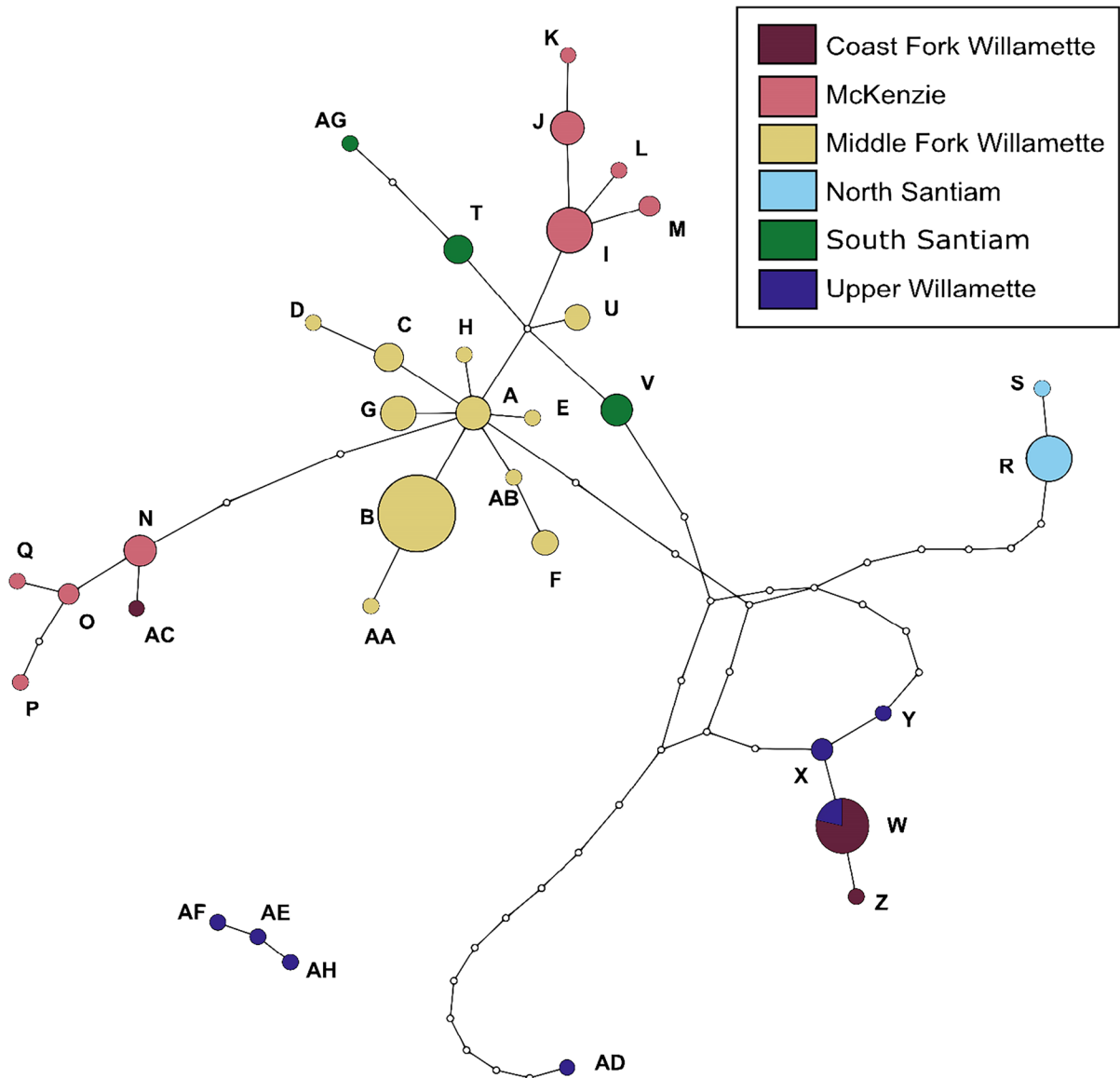


Fig. 5 Statistical parsimony network showing relatedness among concatenated *COI-cyt b* haplotypes of *Lampetra* sp. in headwater subbasins of the Willamette River. Nodes are scaled to the number of individuals observed with that haplotype and are color-coded based on subbasin of capture. Nodes that appear as pie charts show the proportion of individuals that

originated from a particular subbasin. Letters identify the concatenated haplotype represented by each node as listed in Table S4. Note that haplotypes AE, AF, and AH represent three individuals from Muddy Creek represented by the geographically widespread haplotype *cyt b* H39

Willamette River basin (AE, AF, and AH) were so divergent (1.1–1.6%) that they formed a separate network. The Middle Fork Willamette River basin was the most heavily sampled ($n = 59$ *Lampetra* individuals) and the most diverse, representing 11 of the 34 *Lampetra* haplotypes in the concatenated dataset (Table S3). Average divergence among *Lampetra* was 0.13 to 0.79%, and within-basin divergence ranged from 0.01 to 0.66% (Table 2). Except for the Upper Willamette River basin, within-basin divergence was less than that observed among basins. The AMOVA comparing genetic variation among versus within subbasins was significant ($p < 0.001$, d.f. = 133), with 36.0% of variation partitioned among subbasins and 64% within a subbasin.

Discussion

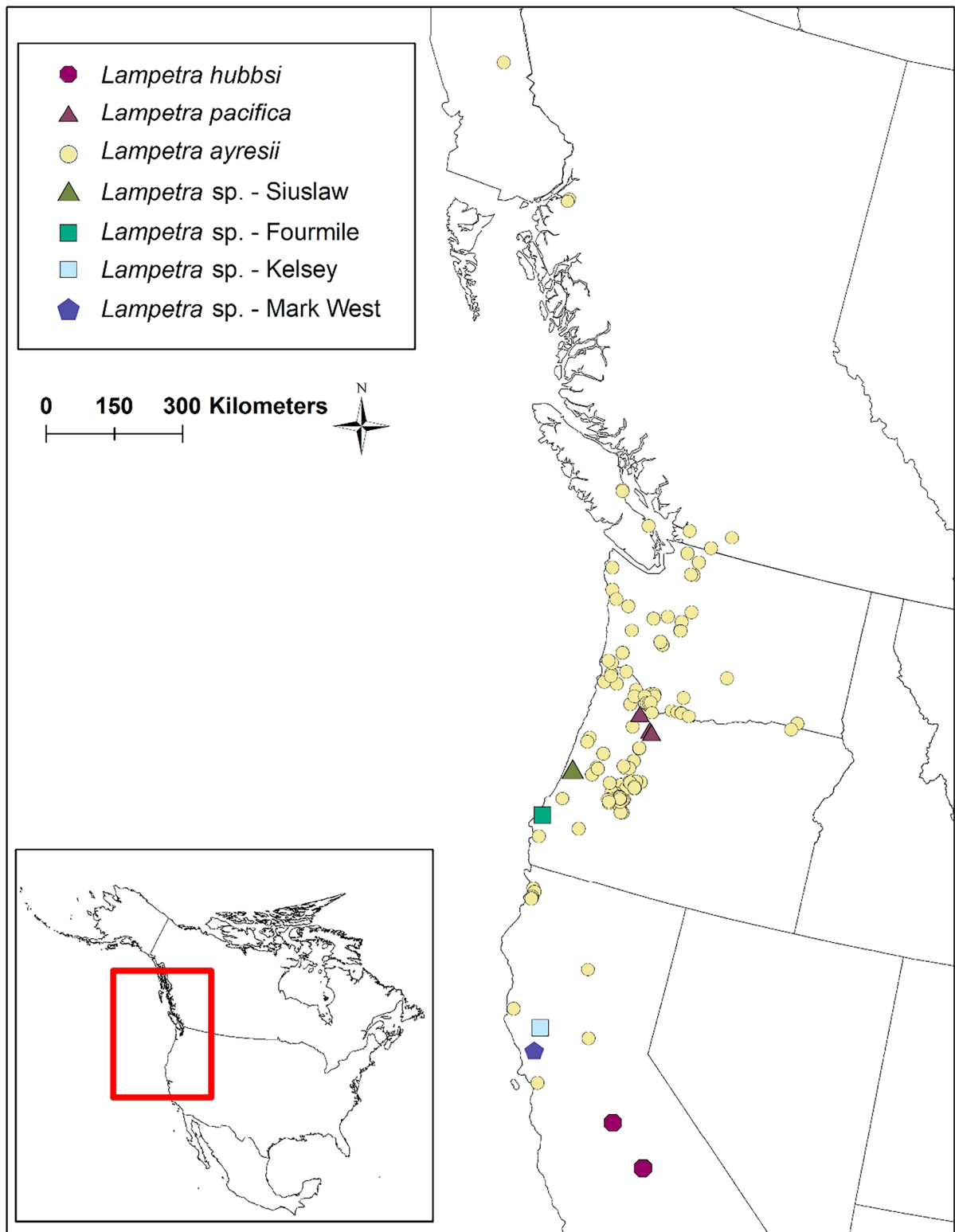
Our phylogenetic analysis comparing the relationships among Petromyzontidae is among the most robust molecular evaluations of this family to date. Our analysis represented every genus and most recognized species of lampreys in the family, included all publicly available sequences, and was repeated for two mitochondrial genes. The analysis reaffirmed Vladykov's (1972) concept of the Petromyzontinae and Lampetrinae subfamilies (see Potter et al. 2015), as well as shortcomings in the present taxonomy also identified by other studies with more limited datasets (Docker et al. 1999; Blank et al. 2008; Lang et al. 2009; Li 2014; White 2014). That three of the genera within Lampetrinae (*Lethenteron*, *Eudontomyzon*, and *Lampetra*) are not monophyletic is thoroughly discussed by others (Lang et al. 2009; Li 2014; Pereira et al. 2021) and could be resolved with reclassification of species among genera, and, in the case of *Lethenteron* sp. S, naming of a new genus (see Yamazaki et al. 2006).

Our study focused on *Lampetra* and the placement of specimens from western North America relative to other congeneric specimens. Like others, using *cyt b* sequences, we observed that *Lampetra* was polyphyletic (Blank et al. 2008; Lang et al. 2009; Li 2014), and these patterns were corroborated by our phylogenetic analysis of *COI* (Fig. 3). Monophyly could be achieved by revising four strongly supported clades: (1) restricting *Lampetra* to members of this genus from Europe and Asia, which includes the type species

for this genus, *Lampetra fluviatilis*, and reassigning *Lethenteron ninae* to this genus; (2) resurrecting *Okkelbergia* as the genus for *Lampetra aepyptera* in eastern North America, as was suggested by Lang et al. (2009); see also Hubbs and Potter (1971) and Vladykov and Kott (1976); (3) retaining the genus *Eudontomyzon* for its current members (save *Eudontomyzon morii* which should be reassigned to *Lethenteron*); and (4) assigning species of *Lampetra* in western North America to a new genus, given that this clade is strongly supported as sister to these three clades in both analyses (as well as *Lethenteron* sensu stricto in the *COI* phylogeny). Although proposing a new genus name according to the Rules of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999) is beyond the scope of this study, formal taxonomic revision is needed to better represent the distinctiveness of western North American *Lampetra*.

Our analyses also favor revisiting species designations within the forms of *Lampetra* from western North America. Four of five tests provided strong support for seven candidate species. These lineages were represented by *Lampetra hubbsi*, *Lampetra pacifica*, four undescribed lineages of *Lampetra*, and one lineage that includes all specimens identified as *Lampetra ayresii* and *Lampetra richardsoni*, as well as a number of unidentified *Lampetra* specimens. Although *Lampetra ayresii* and *Lampetra richardsoni* are considered paired species that can be differentiated based on phenotypic and life history characteristics that develop at metamorphosis (Docker 2009), the species delimitation analysis recognized these as a single species with occasional haplotype sharing and a lack of reciprocal monophyly. Because *Lampetra ayresii* was first described by Guenther in 1870 (see Vladykov and Follet 1958), 95 years prior to the description of *Lampetra richardsoni* (Vladykov and Follet 1965), *Lampetra ayresii* has precedence as the name for members of this clade, in compliance with the principle of priority (Article 23.1) in the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). We regard specimens identified as *Lampetra richardsoni* as a synonymous with *Lampetra ayresii* and consider them to be a life history variant within the *Lampetra ayresii* species complex.

These results support a growing body of evidence that the paired or stem-satellite species concepts which designate species based on feeding type do not



◀**Fig. 6** Location and taxonomic classification of specimens based on species delimitation and specimen assignment analyses. The *L. ayresii* species group includes both anadromous/parasitic and resident/non-parasitic ecotypes, commonly known as western river lamprey and western brook lamprey, respectively

accurately characterize evolutionary lineages warranting recognition as distinct species. Little research has explored mechanisms underlying speciation and evolution of life history diversity in *Lampetra* of western North America, although parasitic individuals consistently appear within a western brook lamprey population on Vancouver Island in British Columbia (Beamish 1985; Beamish et al. 2016), and the report of a migratory *Lampetra* that presumably emerged from a resident population in the Columbia River basin (Jolley et al. 2016) suggests that other west coast *Lampetra* populations may also be polymorphic for feeding type. Research on the paired *Lampetra planeri* (resident, non-parasitic) and *Lampetra fluviatilis* (anadromous, parasitic), both of which are of conservation concern in Europe and at a more local level in some countries (Maitland et al. 2015), may provide insights. Numerous studies across western Europe have demonstrated a polyphyletic relationship between these paired species (Espanhol et al. 2007; Blank et al. 2008; Pereira et al. 2010), and interbreeding is common where they are sympatric (Rougemont et al. 2015; Mateus et al. 2016; Hume et al. 2018). In sympatric populations, *Lampetra planeri* and *Lampetra fluviatilis* more accurately represent a single species with mixed life histories in the early phases of speciation (Rougemont et al. 2017; Kucheryavyy et al. 2016; Mateus et al. 2016; Hume et al. 2018; see also review by Docker and Potter 2019). Genetic divergence between these species is greater where the migratory life history is absent, allowing populations to evolve in relative isolation (Pereira et al. 2010; Bracken et al. 2015; De Cahsan et al. 2020; Rougemont et al. 2021). These studies and our own demonstrate that it is inaccurate to regard these groups as distinct species. Instead, they more accurately represent life history variants of a single species. Similar variation in divergence, hybridization, and life history diversity have been observed across fish taxa including forms of *Oncorhynchus mykiss* (e.g., anadromous steelhead versus freshwater-resident rainbow trout; Docker and Heath 2003), *Oncorhynchus nerka* (e.g.,

anadromous sockeye salmon versus lake-spawning kokanee versus stream-spawning kokanee; Tigano and Russello 2022), western forms of *Rhinichthys cataractae* (longnose and Nooksack dace; Taylor et al. 2015), and many forms of *Gasterosteus aculeatus* (three-spined stickleback; see Gow et al. 2006; Mäkinen et al. 2006) and *Cottus asper* (prickly sculpin; Dennenmoser et al. 2015). In these examples, different common names are sometimes used to distinguish among life history variants of the same species. Similarly, continuing to refer to the migratory/parasitic and resident/non-parasitic forms of *Lampetra ayresii* as western river lamprey and western brook lamprey, respectively, will emphasize the different life histories and ecological requirements of these ecotypes. Recognition of phenotypic and ecological diversity below the species level is important for conservation even if both forms fall under the umbrella of a widespread and abundant species (Docker and Hume 2019).

The four lineages of undescribed *Lampetra* in our species delimitations analyses were originally identified by Boguski et al. (2012). Although they did not perform a formal species delimitation analysis, the authors hypothesized that these lineages from Oregon (Fourmile Creek and Siuslaw River) and California (Mark West Creek and Kelsey Creek) were distinct species based on their high levels of divergence. Furthermore, the authors noted that within Northern Hemisphere lampreys, species diversity, incidence of endemism, and intraspecific genetic variation for broader-ranging taxa are greater at the southern end of their distributions (see Yamazaki et al. 2006; Mateus et al. 2011; Spice et al. 2012), as it is for other amphidromous species in this region (e.g., *Cottus asper*; Young et al. 2022). These patterns are broadly associated with the absence of continental glaciation and its associated climate in the southern portion of their range during the Pleistocene (Reyjol et al. 2009; Griffiths 2010; Su et al. 2022), which has allowed a longer period for divergence of lineages. Based on these patterns, Boguski et al. (2012) hypothesized that the Columbia River may represent the northern boundary of the most genetically divergent lampreys in western North America (see Fig. 6). Furthermore, all four of these candidate species were observed in areas of California and coastal Oregon known for unusually high levels of aquatic endemism (Howard et al. 2015; Markle 2019). Because of limited sampling, it is uncertain whether these four lineages are

more widely distributed or whether other cryptic taxa with similarly limited ranges are present (the latter a pattern typical among species of *Entosphenus* in California; Moyle 2002). Future efforts aimed at recovering additional lineages of *Lampetra* in western North America will likely be the most successful in these regions.

Surprisingly, the distribution of *Lampetra pacifica* also appears extremely limited despite a potential connection to many stream networks. Initially, this species was thought to occupy a number of river basins from Oregon to California (Vladykov 1973) and then later restricted to an undescribed portion of the Columbia River basin (Reid et al. 2011). Specimens of putative *Lampetra pacifica* from the Willamette River basin, including the Upper Willamette and McKenzie subbasins, are represented in museum collections (Table S1). Yet the genetic evaluation of specimens of *Lampetra* from across the Columbia River basin, including the many new samples from the Willamette River basin from the present study, indicates that *Lampetra pacifica* has only been detected in the Clackamas River (the type of location, a tributary to the Willamette River downstream from Willamette Falls) and in Crystal Springs Creek (a tributary 12 km downstream from the mouth of the Clackamas River within the city limits of Portland, Oregon). Given that Reid et al. (2011) did not observe *Lampetra pacifica* in the Tualatin River, whose confluence with the Willamette River is only 5 km upstream from the mouth of the Clackamas River but upstream of Willamette Falls, we hypothesize that the falls constitute the upstream limit of the distribution of *Lampetra pacifica*. The downstream limit remains uncertain. Five museum specimens (four collected in 1963 and one in 2012; Table S1) identified as *Lampetra pacifica* on the basis of morphology were collected in tributaries to the Columbia River downstream of the confluence of the Willamette River. Whether these represent members of the *Lampetra pacifica* lineage is unknown, but further evidence supporting that hypothesis is lacking because individuals genotyped from these and nearby basins have all assigned to *Lampetra ayresii*. Interestingly, the current distribution of this species would have been inundated during the multiple floods associated with ice dam failure of Glacial Lake Missoula and Lake Bonneville draining (O'Connor et al. 2020), suggesting the presence of refugia for this species somewhere

beyond the high- water mark for those events. Where these refugia might have been, whether they remain occupied, and whether *Lampetra pacifica* is found in any other basin warrant further investigation.

Although the species delimitation analysis indicates that *Lampetra ayresii* is the only *Lampetra* species present in our study area in the Willamette River, our extensive sampling effort expanded the known diversity of this species. We observed a total of 21 *cyt b* haplotypes in our study area. Three of these (*cyt b* H03, H04, and H39) were previously observed by Boguski et al. (2012); the other 18 haplotypes were previously unobserved (Fig. 3, Table S3). Based on our analysis, *cyt b* H03 and H04 appear localized to Owens Creek in the Upper Willamette River subbasin. In contrast, *cyt b* H39 was the only haplotype observed in our study area that was also observed in other river basins of western North America. Furthermore, this haplotype was the most widely distributed in the species delimitation dataset, observed in over three dozen individuals ranging from the Farragut River in southeastern Alaska to the Sacramento Delta in central California. Despite its broad distribution, this haplotype was represented by only three individuals captured in Muddy Creek in the Upper Willamette subbasin. Not only was this haplotype rare in our study area, but it was also genetically distinct from all others in the Willamette River basin (Fig. 3, Fig. 5). A similar pattern was observed among members of the *Cottus asper* species complex. Forms of *Cottus asper* in the Marys River (to which Muddy Creek is a tributary) were identical either to forms present in two coastal basins (the Alsea and Yaquina rivers) or to those restricted to the Willamette River basin (Young et al. 2022). These forms of *Cottus asper* and *Lampetra ayresii* that are diverged from others in the Willamette may have arrived via headwater capture at some point in the mid-to-late Pleistocene (Markle 2019), but more recent introductions by anglers are also plausible.

Apart from the divergent Muddy Creek fish, we observed a high level of genetic structure at the subbasin level, with little or no haplotype divergence among specimens from the same basin (Table 2; Table S6; Fig. 5). Similar patterns of genetic structure have been observed among populations of European *Lampetra*, particularly freshwater resident *Lampetra planeri* (Pereira et al. 2010; Bracken et al. 2015; Rougemont et al. 2021). Our results indicate

that *Lampetra ayresii* populations in the Willamette River basin show little evidence of recent dispersal to other subbasins, suggesting that subbasins constitute reasonable units for management and conservation. We acknowledge, however, that the appropriate scale for management in other areas may vary depending on the evolutionary and geological histories of taxa present. For example, both geological and anthropogenic isolation have played a role in structuring *Lampetra* populations in Europe (Pereira et al. 2010; Bracken et al. 2015; De Chasan et al. 2020; Rougemont et al. 2021). Management units for *Lampetra* may encompass several adjacent subbasins in areas with reduced species richness and intraspecific diversity. This may be the case in portions of Washington, British Columbia, and Alaska that were more recently glaciated and likely have less species diversity (Fig. 6; see Boguski et al. 2012). In contrast, smaller conservation units may be required in areas with greater species richness, higher rates of endemism, and in locations where lack of gene flow has led to evolution of divergent lineages (e.g., Pereira et al. 2010; Wang et al. 2020).

In summary, we used mitochondrial gene sequences to address questions related to taxonomic classification, species delimitation, and population structure. Although such data is widely and robustly used in this context, it is not without shortcomings, foremost of which is the separate evolutionary history sometimes exhibited by the nuclear genome (Dellicour and Flot 2018). Further explorations of the patterns we observed, both by consideration of nuclear data (Hess et al. 2013, 2020) and much more extensive and intensive field sampling (Bergsten et al. 2012), are prudent first steps for evaluating our conclusions, in large measure because genetic criteria form part of the basis for designating units of conservation under the US Endangered Species Act (Waples 1991, 1995), Canada's Species at Risk Act (SC 2002), and the United Nations' Convention on Biological Diversity (CBD 2010). Once these units of conservation are identified, tools such as environmental DNA sampling could rapidly and comprehensively resolve issues associated with their distributions (e.g., Young et al. 2022). We believe that a similar stepwise approach applied to other members of Petromyzontidae across the Northern Hemisphere, many of which are also of uncertain taxonomic standing and ambiguous distribution, could prove useful.

Authors' contributions All authors contributed to the study conception and design. Data collection in this study was performed by J. M. Helstab and D. C. Larson. Geographic location information from previously published work was provided by M. F. Docker. Statistical analysis was performed by K. J. Carim and M. K. Young. Data interpretation was performed by all authors. The first draft of the manuscript was written by K. J. Carim, and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

Funding Funding for this work was provided by the US Forest Service.

Data availability All data (genetic sequences) generated as part of study are publicly available through GenBank (ON226748–ON226766; ON419562–ON419582).

Declarations

The authors have no relevant financial or nonfinancial interests to disclose. Margaret F. Docker is the Editor-in-Chief of this journal, but she had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to the Managing Editor and Guest Editors.

Permits and ethics Fish collections were conducted under the Oregon Department of Fish and Wildlife Scientific Taking Permit 4(d), permit # 22632. Sampling efforts were carried out according to the Guidelines for the Use of Fishes in Research published in 2014 by the joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- April J, Mayden RL, Hanner RH, Bernatchez L (2011) Genetic calibration of species diversity among North America's freshwater fishes. *Proc Natl Acad Sci U S A* 108:10602–10607
- Bailey RM (1980) Comments on the classification and nomenclature of lampreys – an alternative view. *Can J Fish Aquat Sci* 37:1626–1629

- Beamish RJ (1987) Evidence that parasitic and nonparasitic life history types are produced by one population of lamprey. *Can J Fish Aquat Sci* 44:1779–1782
- Beamish R, Withler R, Wade J, Beacham T (2016) A nonparasitic lamprey produces a parasitic life history type: the Morrison Creek lamprey enigma. In: Orlov A, Beamish R (eds) *Jawless fishes of the world*, vol 1. Cambridge Scholars Publishing, Newcastle upon Tyne, pp 191–230
- Bergsten J, Bilton DT, Fujisawa T, Elliott M, Monaghan MT, Balke M, Hendrich L, Geijer J, Herrmann J, Foster GN, Ribera I (2012) The effect of geographical scale of sampling on DNA barcoding. *Syst Biol* 61:851–869
- Blank M, Jürss K, Bastrop R (2008) A mitochondrial multigene approach contributing to the systematics of the brook and river lampreys and the phylogenetic position of *Eudontomyzon mariae*. *Can J Fish Aquat Sci* 65:2780–2790
- Bofkin L, Goldman N (2007) Variation in evolutionary processes at different codon positions. *Mol Biol Evol* 24:513–521
- Boguski DA, Reid SB, Goodman DH, Docker MF (2012) Genetic diversity, endemism and phylogeny of lampreys within the genus *Lampetra sensu stricto* (Petromyzontiformes: Petromyzontidae) in western North America. *J Fish Biol* 81:1891–1914
- Bracken FS, Hoelzel AR, Hume JB, Lucas MC (2015) Contrasting population genetic structure among freshwater-resident and anadromous lampreys: the role of demographic history, differential dispersal and anthropogenic barriers to movement. *Mol Ecol* 24:1188–1204
- CBD (Secretariat of the Convention on Biological Diversity) (2010) *Global Biodiversity Outlook 3*, UN Environment Programme, Montréal www.cbd.int/gbo3
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Mol Ecol* 9:1657–1659
- De Cahsan B, Nagel R, Schedina IM, King JJ, Bianco PG, Tiedemann R, Ketmaier V (2020) Phylogeography of the European brook lamprey (*Lampetra planeri*) and the European river lamprey (*Lampetra fluviatilis*) species pair based on mitochondrial data. *J Fish Biol* 96:905–912
- Dellicour S, Flot JF (2018) The hitchhiker's guide to single-locus species delimitation. *Mol Ecol Resour* 18:1234–1246
- Dennenmoser S, Nolte AW, Vamosi SM, Rogers SM (2015) Phylogeography of the prickly sculpin (*Cottus asper*) in north-western North America reveals parallel phenotypic evolution across multiple coastal–inland colonizations. *J Biogeogr* 42:1626–1638
- Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) *Biology, management, and conservation of lampreys in North America*. American Fisheries Society, Bethesda, pp 71–114
- Docker MF, Heath DD (2003) Genetic comparison between sympatric anadromous steelhead and freshwater resident rainbow trout in British Columbia, Canada. *Conserv Genet* 4:227–231
- Docker MF, Hume JB (2019) There and back again: lampreys in the 21st century and beyond. In: Docker MF (ed) *Lampreys: biology, conservation and control*, vol 2. Springer, Dordrecht, pp 527–570
- Docker MF, Potter IC (2019) Life history evolution in lampreys: alternative migratory and feeding types. In: Docker MF (ed) *Lampreys: biology, conservation and control*, vol 2. Springer, Dordrecht, pp 287–409
- Docker MF, Youson JH, Beamish RJ, Devlin RH (1999) Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome b and ND3 gene sequences. *Can J Fish Aquat Sci* 56:2340–2349
- Docker MF, Mandrak NE, Heath DD (2012) Contemporary gene flow between “paired” silver (*Ichthyomyzon unicuspis*) and northern brook (*I. flossor*) lampreys: implications for conservation. *Conserv Genet* 13:823–835
- Espanhol R, Almeida PR, Alves MJ (2007) Evolutionary history of lamprey paired species *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) as inferred from mitochondrial DNA variation. *Mol Ecol* 16:1909–1924
- Fricke R., Eschmeyer WN, Van der Laan R (2022) Eschmeyer's catalog of fishes: genera, species, references. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>. Accessed July 2022
- Gill HS, Renaud CB, Chapleau F, Mayden ML, Potter IC (2003) Phylogeny of living parasitic lampreys (Petromyzontiformes) based on morphological data. *Copeia* 2003:687–703
- Goodman DH, Kinziger AP, Reid SB, Docker MF (2009) Morphological diagnosis of *Entosphenus* and *Lampetra ammocoetes* (Petromyzontidae) in Washington, Oregon, and California. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) *Biology, management, and conservation of lampreys in North America*. American Fisheries Society, Bethesda, pp 223–232
- Gow JL, Peichel CL, Taylor EB (2006) Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. *Mol Ecol* 15:739–752
- Griffiths D (2010) Pattern and process in the distribution of North American freshwater fish. *Biol J Linn Soc* 100:46–61
- Hart MW, Sunday J (2007) Things fall apart: biological species form unconnected parsimony networks. *Biol Lett* 3:509–512
- Hess JE, Campbell NR, Close DA, Docker MF, Narum SR (2013) Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. *Mol Ecol* 22:2898–2916
- Hess JE, Smith JJ, Timoshevskaya N, Baker C, Caudill CC, Graves D, Keefer ML, Kinziger AP, Moser ML, Porter LL (2020) Genomic islands of divergence infer a phenotypic landscape in Pacific lamprey. *Mol Ecol* 29:3841–3856
- Howard JK, Klausmeyer KR, Fesenmyer KA, Furnish J, Gardali T, Grantham T, Katz JVE, Kupferberg S, McIntyre P, Moyle PB, Ode PR, Peek R, Quiñones RM, Rehn AC, Santos N, Schoenig S, Serpa L, Shedd JD, Slusark J et al (2015) Patterns of freshwater species richness, endemism, and vulnerability in California. *PLoS One* 10:e0130710
- Hubbs CL, Potter IC (1971) Distribution, phylogeny and taxonomy. In: Hardisty MW, Potter IC (eds) *The biology of lampreys*, vol 1. Academic Press, London, pp 1–65
- Hume JB, Recknagel H, Bean CW, Adams CE, Mable BK (2018) RADseq and mate choice assays reveal

- unidirectional gene flow among three lamprey ecotypes despite weak assortative mating: insights into the formation and stability of multiple ecotypes in sympatry. *Mol Ecol* 27:4572–4590
- International Commission on Zoological Nomenclature (1999) International code of zoological nomenclature, 4th edn. The International Trust for Zoological Nomenclature, The Natural History Museum, London
- Jenkins JA, Bart HL, Bowker JD, Bowser PR, MacMillan JR, Nickum JG, Rose JD, Sorensen PW, Whitledge GW, Rachlin JW (2014) Guidelines for the use of fishes in research. American Fisheries Society, Bethesda
- Jolley JC, Kovalchuk G, Docker MF (2016) River lamprey (*Lampetra ayresii*) outmigrant upstream of the John Day dam in the mid-Columbia River. *Northwest Nat* 97:48–52
- Kamvar Z, Tabima J, Grunwald N (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281
- Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, Flouri T (2017) Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics* 33:1630–1638
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649
- Kucheryavyy A, Tsimbalov I, Kirillova E, Nazarov D, Zvezdin A, Pavlov D (2016) In: Orlov A, Beamish R (eds) The need for a new taxonomy for lampreys. Jawless fishes of the world, vol 1, Cambridge Scholars Publishing, Newcastle upon Tyne, pp 251–277
- Kucheryavyy A, Tsimbalov I, Kostin V, Nazarov DY, Zvezdin A, Pavlov D (2016) Polymorphism of spawners of the resident form of European river lamprey *Lampetra fluviatilis* (Petromyzontidae). *J Ichthyol* 56:744–751
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Lang NJ, Roe KJ, Renaud CB, Gill HS, Potter IC, Freyhof J, Naseka AM, Cochran P, Pérez HE, Habit EM, Kuhajda BR (2009) Novel relationships among lampreys (Petromyzontiformes) revealed by a taxonomically comprehensive molecular data set. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Bethesda, pp 41–55
- Li Y (2014) Phylogeny of the lamprey genus *Lethenteron* Creaser and Hubbs 1922 and closely related genera using the mitochondrial cytochrome *b* gene and nuclear gene introns. Master's thesis, University of Manitoba, Winnipeg
- Lorion CM, Markle DF, Reid SB, Docker MF (2000) Redescription of the presumed-extinct Miller Lake lamprey, *Lampetra minima*. *Copeia* 2000:1019–1028
- Maitland PS, Renaud CB, Quintella BR, Close DA, Docker MF (2015) Conservation of native lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 1. Springer, Dordrecht, pp 375–428
- Makinen HS, Cano JM, Merila J (2006) Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. *Mol Ecol* 15:1519–1534
- Markle DF (2019) Drainage evolution and freshwater fish zoogeography in coastal Oregon and Washington. *Northwest Nat* 100:71–89
- Mateus CS, Almeida PR, Mesquita N, Quintella BR, Alves MJ (2016) European lampreys: new insights on postglacial colonization, gene flow and speciation. *PLoS One* 11:e0148107
- McPhail JD, Lindsey CC (1970) Freshwater fishes of northwestern Canada and Alaska. *Bulletin of the Fisheries Research Board of Canada* 173:1–381
- Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol* 30:1188–1195
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 37:1530–1534
- Moyle PB (2002) Inland fishes of California: revised and expanded. University of California Press, Berkeley
- Nixon KC, Wheeler QD (1990) An amplification of the phylogenetic species concept. *Cladistics* 6:211–223
- O'Connor JE, Baker VR, Waitt RB, Smith LN, Cannon CM, George DL, Denlinger RP (2020) The Missoula and Bonneville floods—a review of ice-age megafloods in the Columbia River basin. *Earth-Sci Rev* 208:103181
- Pereira AM, Robalo JI, Freyhof J, Maia C, Fonseca JP, Valente A, Almada VC (2010) Phylogeographical analysis reveals multiple conservation units in brook lampreys *Lampetra planeri* of Portuguese streams. *J Fish Biol* 77:361–371
- Pereira AM, Levy A, Vukic J, Sanda R, Levin BA, Freyhof J, Geiger M, Choleva L, Francisco SM, Robalo JI (2021) Putting European lampreys into perspective: a global-scale multilocus phylogeny with a proposal for a generic structure of the Petromyzontidae. *J Zool Syst Evol Res* 59:1982–1993
- Potter IC (1980) The Petromyzoniformes with particular reference to paired species. *Can J Fish Aquat Sci* 37:1595–1615
- Potter IC, Gill HS, Renaud CB, Haoucher D (2015) The taxonomy, phylogeny, and distribution of lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 1. Springer, Dordrecht, pp 35–73
- Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol Ecol* 21:1864–1877
- Puillandre N, Brouillet S, Achaz G (2021) ASAP: assemble species by automatic partitioning. *Mol Ecol Resour* 21:609–620
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Ratnasingham S, Hebert PD (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS One* 8:e66213
- Reid SB, Boguski DA, Goodman DH, Docker MF (2011) Validity of *Lampetra pacifica* (Petromyzontiformes: Petromyzontidae), a brook lamprey described from the lower Columbia River Basin. *Zootaxa* 3091:42–50

- Renaud CB (2011) Lampreys of the world. An annotated and illustrated catalogue of lamprey species known to date. Food and Agriculture Organization of the United Nations, Rome
- Robins CR, Bailey RM, Bond CE, Brooker JR, Lachner EA, Lea RN, Scott WB (1991) A list of common and scientific names of fishes from the United States and Canada. American Fisheries Society, Bethesda
- Rougemont Q, Gaigher A, Lasne E, Côte J, Coke M, Besnard AL, Launey S, Evanno G (2015) Low reproductive isolation and highly variable levels of gene flow reveal limited progress towards speciation between European river and brook lampreys. *J Evol Biol* 28:2248–2263
- Rougemont Q, Dolo V, Oger A, Besnard A, Huteau D, Coustellet M, Perrier C, Launey S, Evanno G (2021) Riverscape genetics in brook lamprey: genetic diversity is less influenced by river fragmentation than by gene flow with the anadromous ecotype. *Heredity* 126:235–250
- Santos AM, Cabezas MP, Tavares AI, Xavier R, Branco M (2015) tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics* 32:627–628
- SC (Statutes of Canada) (2002) Species at Risk Act. <https://www.laws-lois.justice.gc.ca/PDF/S-15.3.pdf>
- Spice EK, Goodman DH, Reid SB, Docker MF (2012) Neither philopatric nor panmictic: microsatellite and mtDNA evidence suggests lack of natal homing but limits to dispersal in Pacific lamprey. *Mol Ecol* 21:2916–2930
- Su G, Tedesco PA, Toussaint A, Villéger S, Brosse S (2022) Contemporary environment and historical legacy explain functional diversity of freshwater fishes in the world rivers. *Glob Ecol Biogeogr* 31:700–713
- Taylor EB, Harris LN, Spice EK, Docker MF (2012) Microsatellite DNA analysis of parapatric lamprey (*Entosphenus* spp.) populations: implications for evolution, taxonomy, and conservation of a Canadian endemic. *Can J Zool* 90:291–303
- Taylor EB, McPhail JD, Ruskey JA (2015) Phylogeography of the longnose dace (*Rhinichthys cataractae*) species group in northwestern North America — the Nooksack dace problem. *Can J Zool* 93:727–734
- Tigano A, Russello MA (2022) The genomic basis of reproductive and migratory behaviour in a polymorphic salmonid. *Mol Ecol* 31:6588–6604. <https://doi.org/10.1111/mec.16724>
- U. S. Fish and Wildlife Service (2004) Endangered and threatened wildlife and plants; 90-day finding on a petition to list three species of lampreys as threatened or endangered. *Federal Register* 69:77158–77167
- United States Geological Survey (2004) National Hydrography Dataset. United States Department of the Interior, [Reston, Virginia.] <https://www.usgs.gov/national-hydrography/national-hydrography-dataset>
- Vladykov VD (1972) Sous-division en trois sousfamilles des lampreies de l'hémisphère nord de la famille Petromyzonidae. *Acfas* 39:148
- Vladykov VD (1973) *Lampetra pacifica*, a new nonparasitic species of lamprey (Petromyzontidae) from Oregon and California. *J Fish Res Board Can* 30:205–213
- Vladykov VD, Follett WI (1958) Redescription of *Lampetra ayresii* (Günther) of western North America, a species of lamprey (Petromyzontidae) distinct from *Lampetra fluvialis* (Linnaeus) of Europe. *J Fish Res Board Can* 15:47–77
- Vladykov VD, Follett WI (1965) *Lampetra richardsoni*, a new nonparasitic species of lamprey (Petromyzonidae) from western North America. *J Fish Res Board Can* 22:139–158
- Wang CJ, Schaller HA, Coates KC, Hayes MC, Rose RK (2020) Climate change vulnerability assessment for Pacific lamprey in rivers of the western United States. *J Freshw Ecol* 35:29–55
- Waples RS (1991) Pacific salmon, *Oncorhynchus* spp., and the definition of “species” under the Endangered Species Act. *Mar Fish Rev* 53:11–22
- Waples RS (1995) Evolutionary significant units and the conservation biological diversity under the Endangered Species Act. In: Nielsen JL (ed) *Evolution and the aquatic ecosystem: defining unique units in population conservation*. American Fisheries Society, Bethesda, pp 8–27
- Ward RD, Hanner R, Hebert PDN (2009) The campaign to DNA barcode all fishes, FISH-BOL. *J Fish Biol* 74:329–356
- White MM (2014) Intraspecific phylogeography of the American brook lamprey, *Lethenteron appendix* (DeKay, 1842). *Copeia* 2014:513–518
- Wydoski RS, Whitney RR (2003) *Inland fishes of Washington*, 2nd edn. American Fisheries Society, Bethesda
- Yamazaki Y, Yokoyama R, Nishida M, Goto A (2006) Taxonomy and molecular phylogeny of *Lethenteron* lampreys in eastern Eurasia. *J Fish Biol* 68:251–269
- Young MK, Smith R, Pilgrim KL, Isaak DJ, McKelvey KS, Parkes S, Egge J, Schwartz MK (2022) A molecular taxonomy of *Cottus* in western North America. *West N Am Nat* 82:307–345
- Zanandrea G (1959) Speciation among lampreys. *Nature* 184:360
- Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29:2869–2876

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.